EXHIBIT A

Supporting Experimental Data

We determined whether the three candidate peptides, shown in Table 1, would attenuate Aβ's SOD-like activity in a DCF assay and additionally determined the species specific nature of this modification.

Table 1 Identification of phage display peptides that bind human Aβ1-42

Peptide sequence/length Name	Number of identical sequences after Round 4	Number of identical sequences after Round 5
TNPNRRNRTPQMLKR[#] 15-mer Peptide A (SEQ ID NO:3)	3/20	5/20
PLPQML[#] 6-mer Peptide B (SEQ ID NO:2)	7/20	8/20
MTMPTM 6-mer Peptide C (SEQ ID NO:1)	2/20	5/20

[#] Share sequence homology.

To achieve this, Peptide A (SEQ ID NO:3) (Figure 1a), Peptide B (SEQ ID NO:2) (Figure 1b) and Peptide C (SEQ ID NO:1) (Figure 1c) were added to 200 nM of each Aβ species (human Aβ1-42, rat Aβ1-42 and human reverse Aβ42-1) in the presence of 400 nM Cu^{2+}) at concentrations of 100, 200, 400, 1000 and 2000 nM.

The addition of Peptide A (Figure 1a) significantly and specifically reduced H_2O_2 production by human Aβ1-42 (One way ANOVA; $F_{(5,84)} = 15.653$, $P < 0.001$), but had no effect on either human reverse Aβ42-1 or rat Aβ1-42. Additionally, it produced negligible native H_2O_2. Significant reductions in H_2O_2 levels were observed at 100, 200 and 400 nM ($P < 0.001$) and to a lesser extent at 1000 nM ($P < 0.05$). Similar results were found for Peptides B and C. The above findings, taken together, indicate that all three candidate peptides possess the ability to modify Aβ1-42's SOD-like activity. However, of these, Peptide A is the most promising, based on its efficacy and specificity in reducing human Aβ1-42's SOD-like activity.

We also determined whether the three candidate peptides protected against Aβ-induced neuronal toxicity as assessed by the LDH and MTT assays. The LDH assay measures the activity of LDH enzyme released from cells as a result of comprised plasma membrane integrity. The LDH release induced by Aβ1-42 action on M17 cells was effectively attenuated in the presence of Peptide A at concentrations ranging from 5-100 μM. (Figure 2a; One way ANOVA; $F_{(5,53)} = 30.847$, $P < 0.001$). Peptide A completely blocked Aβ1-42 induced LDH release at a concentration of 50 μM. Neither of the two 6-mer peptides significantly reduced

LDH levels as compared to Aβ only (Figure 2; One way ANOVA; MTM, $F_{(4,45)}$ = 1.126, P = 0.356; PLP, $F_{(4,45)}$ = 3.710, $P < 0.05$). Peptides B and C did not alter Aβ1-42 induced LDH release at the concentrations tested (Fig 2b and 2c respectively).

We then further assessed the ability of the most promising peptide, Peptide A, to protect against Aβ-induced toxicity using the MTT assay. The MTT assay is commonly used to determine viability and/or metabolic activity of cells. Assessing the effect of the candidate peptides on Aβ toxicity in this assay is particularly relevant, as it has been shown that the addition of catalase (an enzyme that consumes H_2O_2) blocks Aβ-induced neurotoxicity, demonstrating that H_2O_2 is directly responsible for Aβ's toxic effects in this assay.

M17 cells incubated for 6 h with 10 μM human Aβ1-42 in Locke's buffer resulted in a significant loss of viability/metabolic activity when compared to untreated controls. The addition of Peptide A at concentrations ranging from 1-100 μM, to human Aβ1-42 treated cells produced a dose-dependent increase in the percentage of metabolically active cells (Figure 3; results up to 5 μM shown). These results indicate that this peptide can effectively block Aβ1-42 induced neurotoxicity in the M17 cells. Similar experiments were carried out on Peptides B and C (data not shown). However, whilst there was some effect from these peptides, overall they were found to be much less effective.

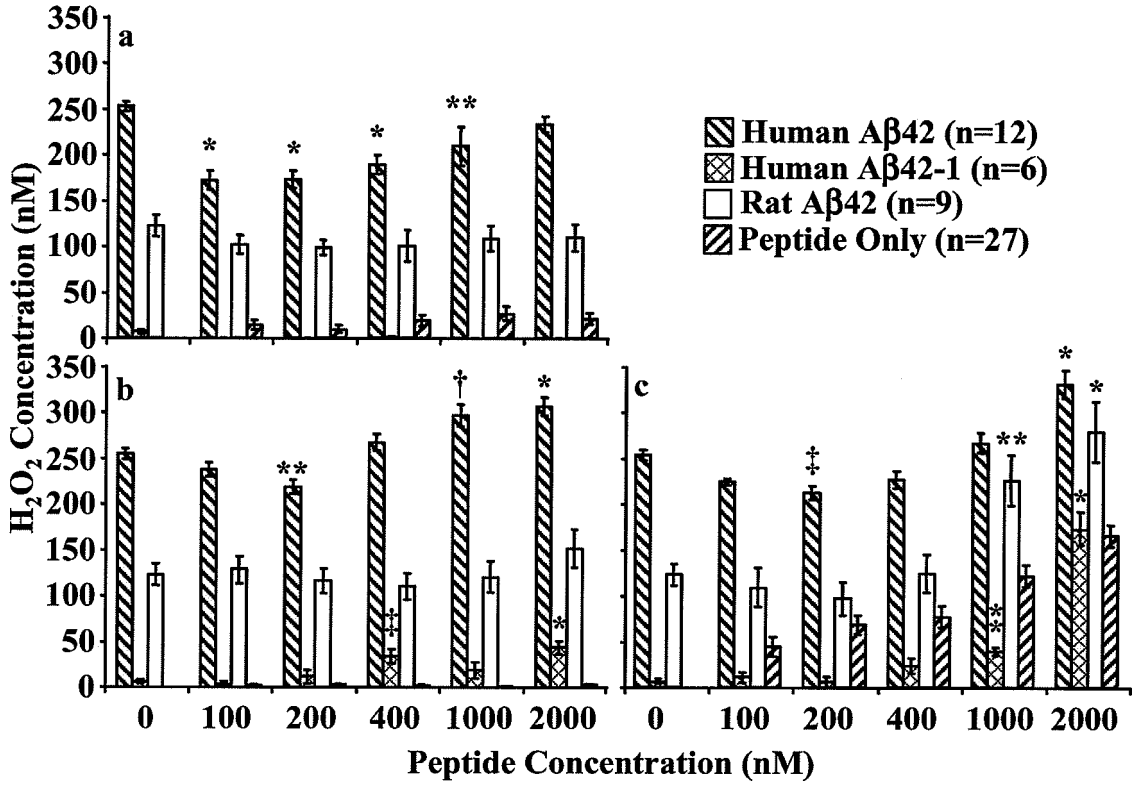


Figure 1. Dose-response effect of the three candidate peptides, Peptide A (a), Peptide B (b), and Peptide C (c), on H_2O_2 production by Aβ42 species. Data are presented as means ± SEM. For statistical analyses, One-way ANOVA with post-hoc analysis (Tukey HSD), correcting for multiple comparisons, was performed. *$p < 0.001$, ** $p < 0.05$, † $p < 0.005$ and ‡ $p < 0.01$, significantly different compared to the respective Aβ species only (i.e. no peptide).

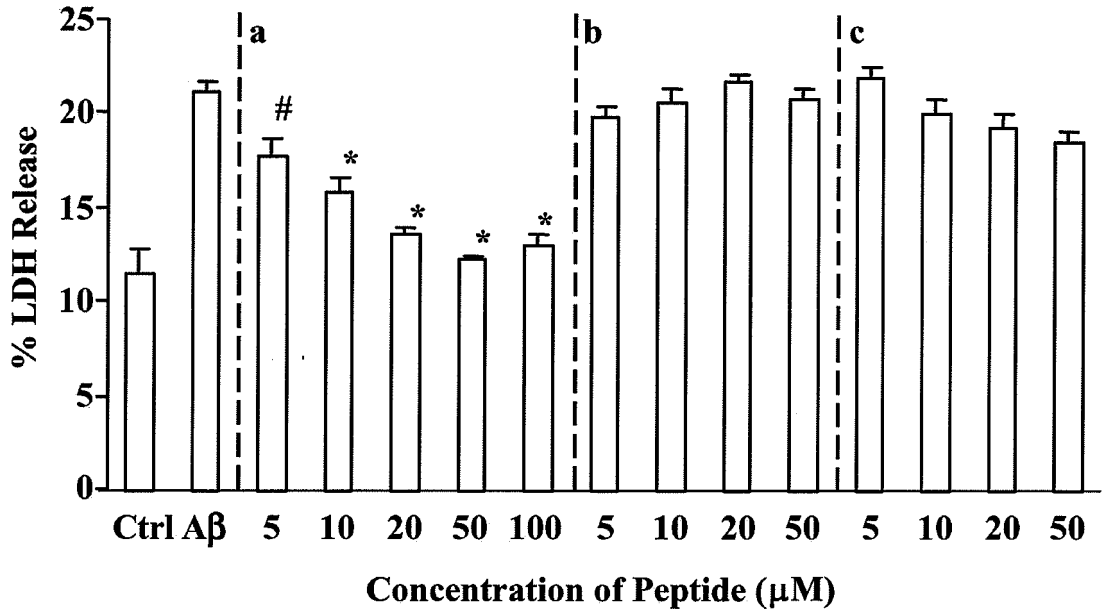


Figure 2. Percentage of lactate dehydrogenase (LDH) activity released by untreated and treated M17 cells. Cells were treated with 10 μM human Aβ1-42, for 120 hours, in the absence or presence of varying doses of the three candidate peptides, Peptide A (a), Peptide C (b), Peptide B (c). Data are presented mean ± SEM (n = 9). For statistical analyses, One-way ANOVA with post-hoc analysis (Tukey HSD), correcting for multiple comparisons, was performed. *$p < 0.001$ and #$p < 0.01$, significantly different compared to Aβ1-42 only treated cells.

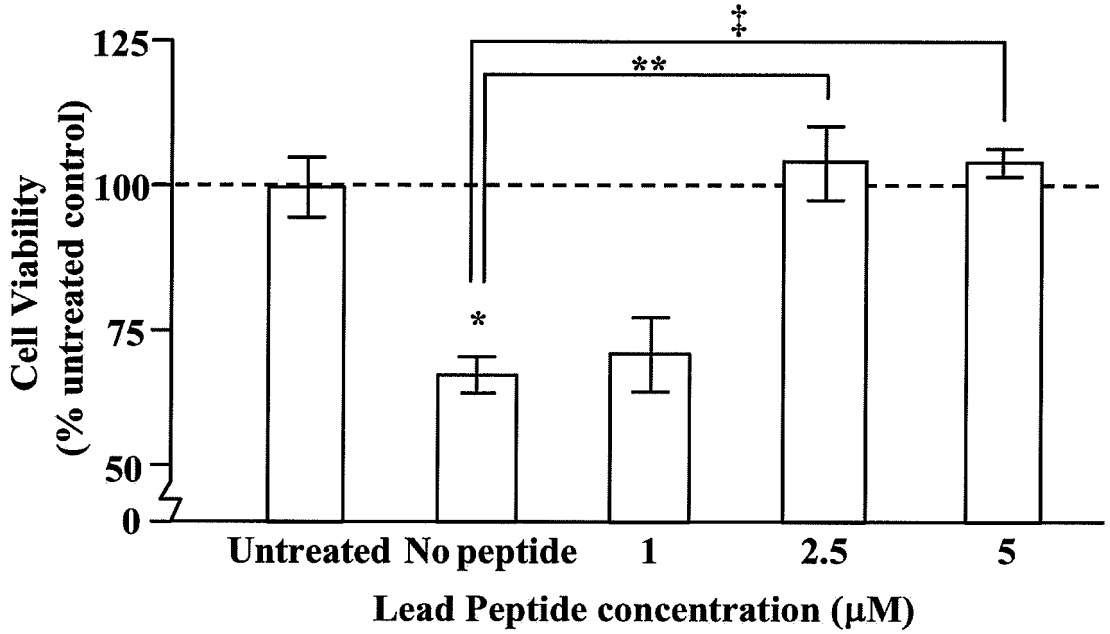


Figure 3 Cell viability of M17 cells treated with human Aβ1-42 in the presence of the most promising peptide, Peptide A. M17 cells were treated for 6 hours at 37°C with soluble human Aβ1-42 (10 μM) in the absence or presence of Peptide A (1, 2.5 or 5 μM). 10 μM soluble human Aβ1-42 in Lockes buffer results in a 35% reduction of cell viability. Data is expressed as a percentage of untreated controls and represents SEM of three experiment performed in sextuplicate (n=18). The addition of the Peptide A, significantly inhibited toxicity induced by soluble human Aβ1-42 providing maximum protection at a concentration of 2.5 μM. Data are presented as means ± SEM. For statistical analyses, One-way ANOVA with post-hoc analysis (Tukey HSD), correcting for multiple comparisons, was performed. ** $P < 0.05$, ‡ $P < 0.01$ versus No peptide, * $P < 0.001$ versus untreated.

Supplemental Data – Part 2

Acute Aβ Induced Neurodegeneration Rat Model

A reliable animal model of neurodegeneration may be induced by injections of Aβ42 into the rat hippocampus. This Aß model of Alzheimer's disease is an ideal tool for the *in vivo* investigation of compounds with neuroprotective potential.

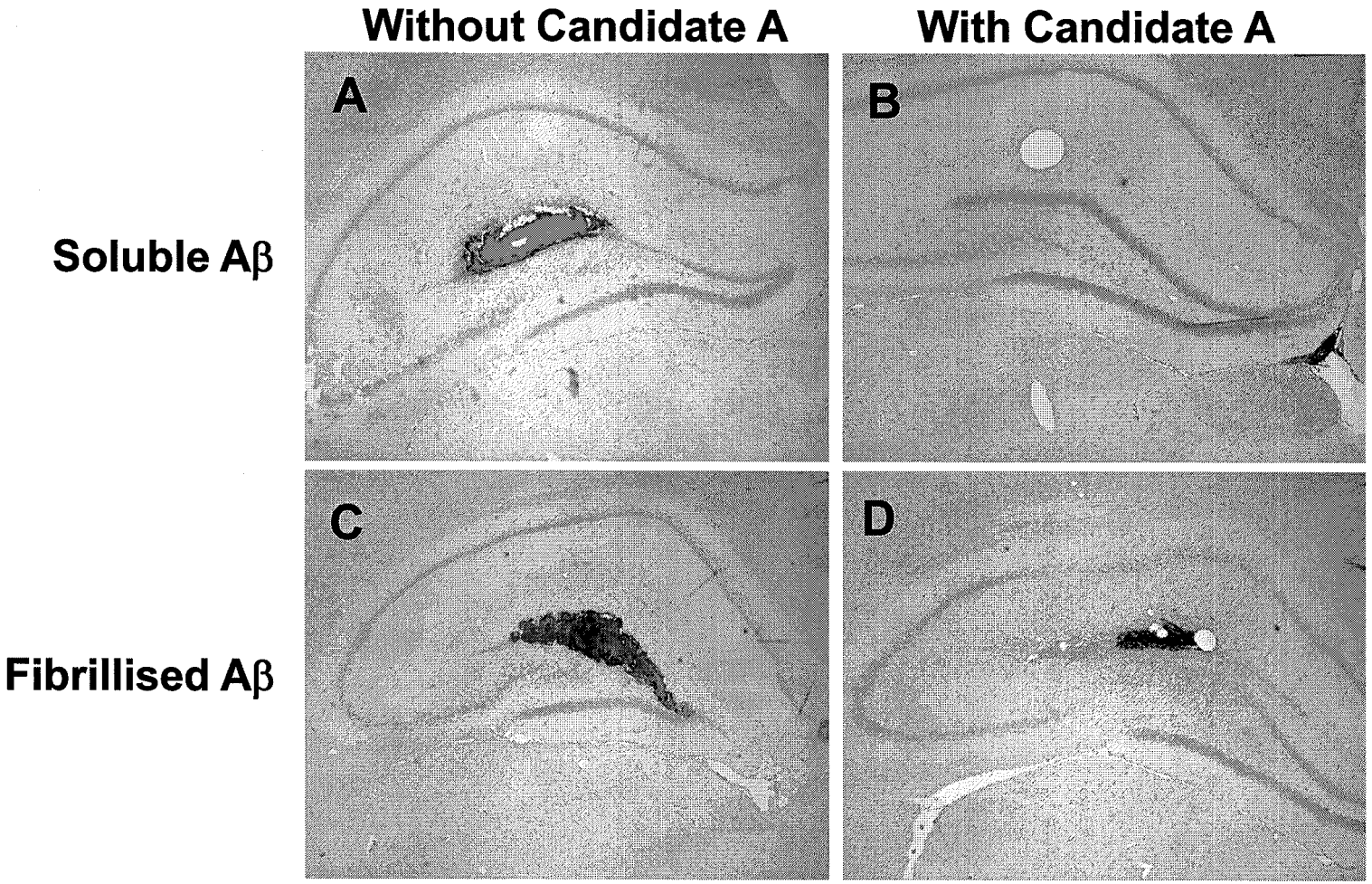
Injections of Aβ42 induce degeneration of neurons of the hippocampal CA1 subfield, and produce neurochemical and immunohistochemical changes resembling those occurring in Alzheimer's disease [Kowall et al., PNAS USA. (1991); Miguel-Hidalgo et al., Eur Neuropsychopharmacol. (1998); Alvarez et al., J Neurosci. (1998)].

The present data results from an experiment to determine the effect of treatment of such a model with Candidate molecule A (15-mer: Thr-Asn-Pro-Asn-Arg-Arg-Asn-Arg-Thr-Pro-Gln-Met-Leu-Lys-Arg).

It was found that 15mer increases the clearance of Aβ from the brain, suggesting that the peptide has two modes of action:

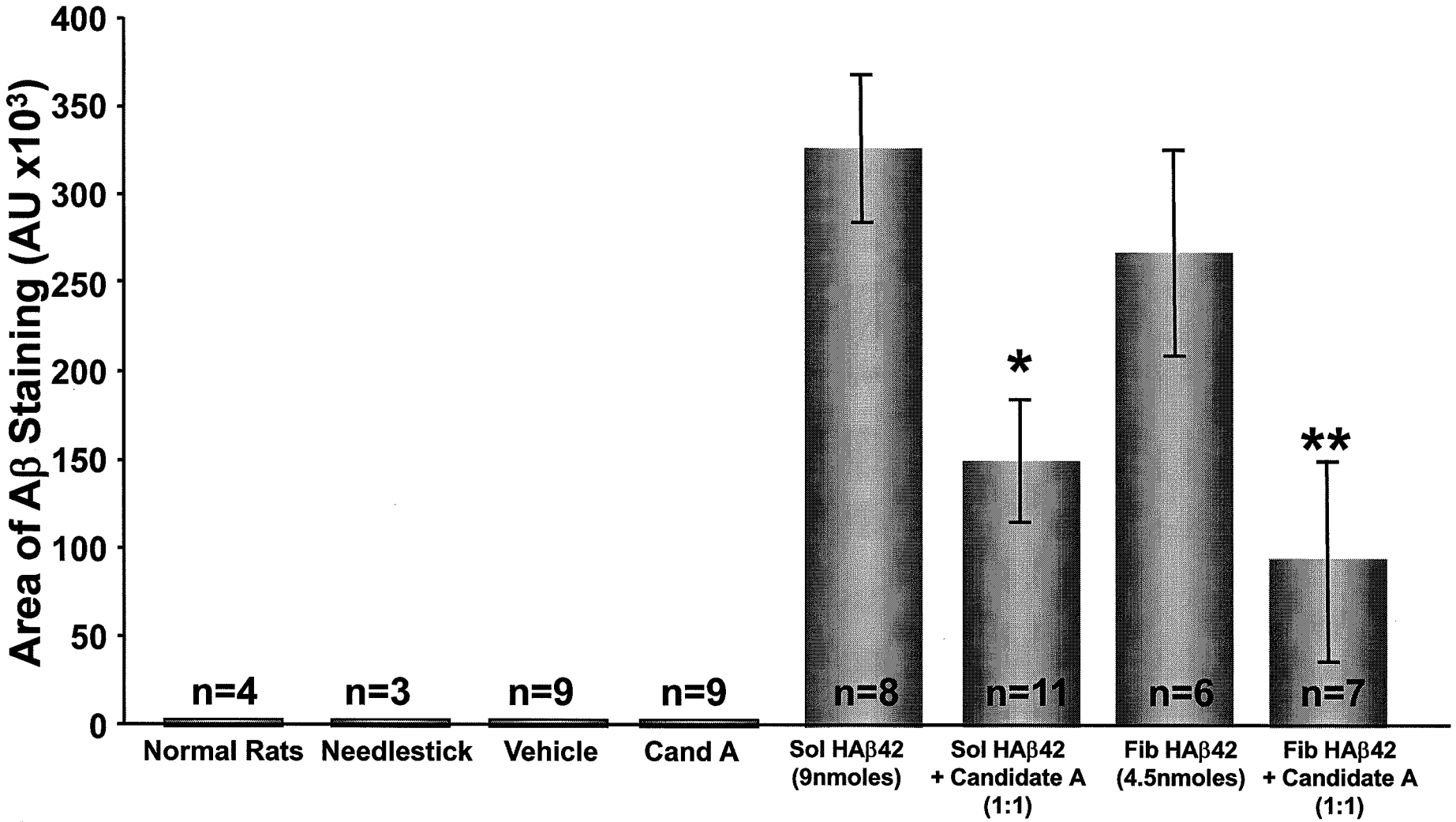
1. neuroprotection by prevention of Aβ toxicity; and
2. facilitating Aβ clearance from the brain.

Aβ Staining of Rat Brain Injected With Human Aβ42 ± Candidate A Peptide



alzhyme

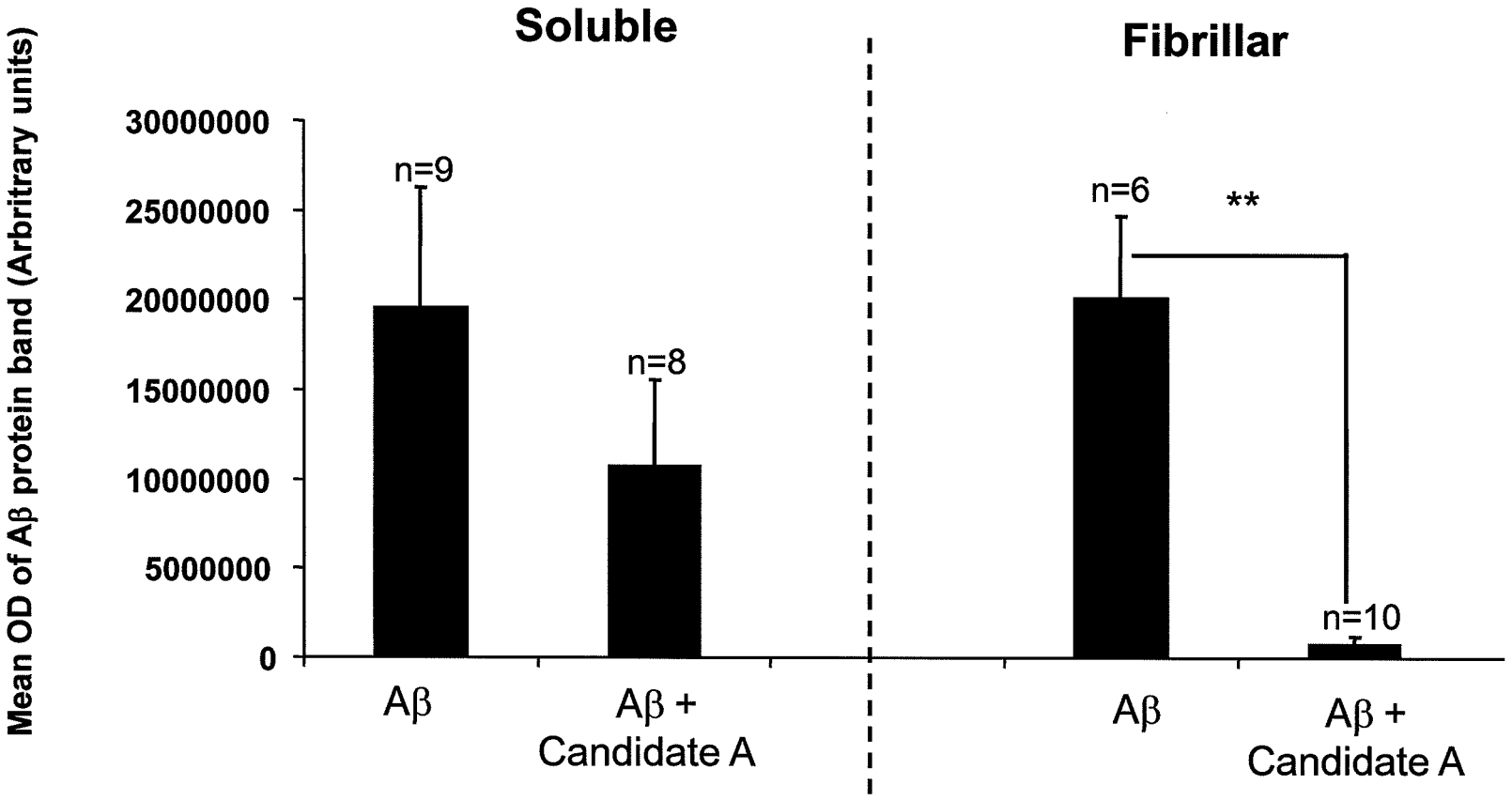
Levels of Aβ Staining in the Hippocampus of Rats



ANOVA: $F_{(3,28)}$=5.271, P<0.005; * t=-2.957, p<0.05† vs Soluble HAβ42, ** t=-2.427, p<0.05† vs Fibrillar HAβ42
†Bonferroni correction applied for multiple comparisons

alzhyme

Aβ Levels in the Hippocampus of Injected Rats



*p<0.01 Significantly decreased from fibrillar Aβ by one way ANOVA with post-hoc (Tukey HSD)

alzhyme